

Picking Clones

1. Transfect 5 ug of the desired plasmid to the desired adherent cell line.
2. Transfect in parallel GFP and carrier DNA (total of 5 ug).
3. 48hrs after transfection check for transfection efficiency.
4. Taking into account the transfection efficiency split cells. 2 plates with 3×10^4 , 1×10^5 , 3×10^5 green cells.
5. 48hrs after splitting cells, add drug for selection.
6. 10 days after adding the selection drug, look for those colonies that have about 100 cells and circle them.
7. Let them grow so that there about 1000 cells.
8. 2 weeks after adding the selection drug, pick the colonies.
9. If you look under the plate, colonies look like gray circles. Under the microscope, they almost fill up the field of view but do not exceed it.

To pick colonies

1. Circle those colonies that will be picked.
2. Take off the media and add PBS to wash.
3. Take off the PBS.
4. Take the tweezers :
rinse in 70% ethanol
Shake off the excess
Rinse in PBS
Shake off the excess
5. Grab a small piece of whatman paper that is being soaked in trypsin and place it on top of the colony of cells.
6. Repeat step 4 and 5 for each colony.
7. Look under the microscope. Cells surrounding the whatman paper should look round if they are being ready to be picked.
8. Add 2 mL of media to each well in the 6-well plate. Media should contain all the antibiotics.
9. Take the tweezers and wash in ethanol and PBS before grabbing the whatman paper and dropping it in a well.
10. Repeat the wash of the tweezers between colonies of cells that are picked.
11. Look at the plate in the air or under the microscope and make sure that most of the cells within the circle have been removed.
12. Pipet up and down each well so that you remove most of the cells still on the whatman paper.
13. Look at each well to make sure that there are some cells in it.
14. Leave the whatman paper in the well.
15. Check the wells 4 or 5 days later. Cells should be spreading and growing.
16. Different wells will grow at different rates. Expand the ones that grow fast first and let the other ones keep on growing.
17. Also keep a back up.